DNA methylation

Dispatch

DNA Methylation: Mega-Year Inheritance With the Help of Darwin

DNA methylation in a fungal pathogen has persisted for millions of years without the enzyme that can efficiently add methyl groups *de novo*. This spectacular example of 'epigenetic' inheritance is explained by a super-efficient maintenance enzyme plus natural selection.

Adrian Bird

~~A~~ defining feature of 'epigenetic' marks is that they should persist through cell division, so that any information encoded by the epigenomic pattern is inherited by the daughter cells. Methylation of cytosine bases in DNA potentially fulfils this requirement, as in many organisms this chemical modification occurs in the self-complementary sequence CG, which suggests an inheritance mechanism [1,2]. Cytosine becomes methylated after DNA has already been synthesised, and consequently replication of the genome produces hemi-methylated CG pairs which are specifically recognised and completed by a 'maintenance' DNA methyltransferase (DNMT). An ideal maintenance enzyme would be totally inactive on non-methylated CG pairs, exclusively copying the previous pattern of CG methylation onto the newly synthesised DNA strand with perfect accuracy [3]. The mammalian maintenance enzyme, even with the assistance of its specificity factor UHRF1 [4,5], is only moderately successful in this endeavour, as the fidelity of maintenance is imperfect [6]. This means that any pattern of CG methylation tends to degrade fairly rapidly in a proliferating cell population, unless reinforced by enzymes that refresh it by adding missing methyl groups. As the fidelity of maintenance methylation consistently fails to approach the extra-ordinary precision of DNA replication it has become accepted that methylation will usually be maintained through collaboration between error-prone maintenance and *de novo* DNMTs. This presumption is thrown

into question, however, by the recent report [7] that a fungal DNA methylation pattern has resisted degradation in the apparent absence of a *de novo* methyltransferase, not just for multiple generations, but for many millions of years.

The organism concerned is *Cryptococcus neoformans*, a fungal pathogen often associated with human HIV infection. In this organism, DNA methylation is overwhelmingly found in the dinucleotide CG at transposon-derived repeats which cluster near the centromeres [8]. DNA methylation is essential for efficient infection, perhaps because it prevents these elements from causing genetic damage through transposition [9]. However, the conventional assumption that the stability of the DNA methylation would be due to maintenance and *de novo* DNA methyltransferase acting in concert is undermined by the observation that *C. neoformans* only has one candidate methyltransferase, DMT5, and this resembles the maintenance enzyme found in other species [8]. If this enzyme is specialised for maintenance, why does DNA methylation not gradually degrade in the absence of an enzyme that keeps it topped up? In this comprehensive study, the authors combine biochemistry, genetics and comparative evolutionary biology to explain this phenomenon.

Like some plant DNMTs, DMT5 is ATP-dependent and binds to the 'repressive' histone mark H3K9me. It also interacts with UHRF1, which helps targeting to hemi-methylated CG sites [10]. The authors verify its credentials as a maintenance enzyme *in vitro* by showing that the purified protein completes hemi-methylated CG sites with high fidelity, but has no detectable activity on unmethylated DNA. They then try to detect significant *de novo* methylation activity in several ways, but without success. Most conclusively, they genetically disrupt the DMT5 gene leading to loss of DNA methylation and then reconstitute it using homologous recombination to restore native levels of the protein. Using this and other experimental approaches, putting back the enzyme failed to reinstate DNA methylation, implying that DMT5 cannot efficiently add methyl groups to unmethylated DNA. In occasional experiments, however, barely detectable levels of mC were

apparent, hinting at minimal *de novo* activity of DMT5. To put numbers on the rates of gain and loss the authors conducted experimental evolution starting from single clones. Using whole genome bisulfite analysis to determine the rates of gain and loss of methylation at single nucleotide resolution, they observed that methylation loss occurred with a frequency close to 10^{-6} per CG site per generation, which is indeed an extraordinarily low error rate. By some margin this is the most efficient maintenance DNMT yet detected. Importantly, *de novo* methylation also occurred at a rate that could be measured. Crucially, the addition of new methyl groups was 20 times slower than the loss of methylation due to maintenance errors. If maintenance had been perfect, even this extremely low *de novo* rate would lead to a relentless increase in overall DNA methylation with time, as every reluctantly added methyl group would be maintained and spread. As the rate of loss events in fact exceeds the rate of gain events, however, the system cannot be at equilibrium. How then does this organism maintain a constant methylome?

To better understand how *C. neoformans* ended up with a maintenance DNMT but no obvious *de novo* enzyme, the authors examined evolutionarily related species. The closest relative, *Cryptococcus gattii*, is highly informative, as it has inactivating mutations in all its transposable elements and at the same time has lost all potential DNMTs including DMT5 [11]. This suggests that natural selection is at work; clones that lose all active transposons no longer need DNA methylation to restrain them and so can lose DMT5 with impunity. More distantly related fungi do have a DMT5 ortholog, but also an enzyme which these authors christen 'DMTX'. This, they hypothesised, may be the missing *de novo* enzyme (Figure 1). Fulfilling this expectation, expression of DMTX in DNA methylation-deficient *C. neoformans* leads to a gradual increase in mCG, particularly in repetitive elements, although the reason for specificity is unclear. Comparative analysis allows the authors to put in perspective the achievement of DMT5 in maintaining *C. neoformans* methylation, as DMTX appears to have been lost over 50 million years ago.

There is evidently a big discrepancy between the fidelity of DMT5 and retention of DNA methylation for millions of years. Even with its unprecedentedly low error rate, *C. neoformans* might only be expected to keep methylation levels intact for a century or so. How then can stability of the DNA methylome over millions of years be accounted for? One possibility is that there are periodic saltatory *de novo* methylation events. A process that can provoke transposon silencing involving DNA methylation is meiosis [12], but sexual reproduction of DNA methylation-deficient fungi expressing DMT5 did not restore CG methylation at peri-centromeric repeats or elsewhere. Given the often invoked (but rarely validated) relationship between the environment and the epigenome, the authors attempted, without success, to trigger DNA methylation with stress. It is of course impossible to prove that DMT5 or another protein encoded by the genome never springs into action as a *de novo* DNMT, but the available data offers no support for this idea.

Although the maintenance DNMT in this fungus is a lot better at maintaining CG methylation than mammalian equivalents, it is still nowhere near good enough to explain retention of methylation for millions of years. Based on comparative analysis among transposable element sequences in related species the authors show persuasive evidence that the missing ingredient is Darwinian selection (Figure 1). Lose methylation and your descendants are doomed, presumably because transposition is mutagenic or centromere function is adversely affected. Given the inevitable attrition caused by rare methylation loss, this would still require some way of topping up mC levels beyond the very low *de novo* activity that they measure. Intriguingly, the authors suggest that the phenomenon of gene conversion may be involved, whereby the methylated strand of one element invades the DNA of a non-methylated recipient element, as previously reported in a different fungus [13]. This will automatically create a hemi-methylated intermediate; in other words, a perfect DMT5 substrate. The end result would be methylation of a previously unmethylated element due to genetic recombination (arguably a genetic, rather than an epigenetic process). Because of the need for selection to explain persistent methylation, the authors refer to this as “Darwinian epigenome

inheritance". So, while at first sight *C. neoformans* appears to represent an almost miraculous example of quasi-eternal mCG maintenance, the thorough study by Catania and colleagues shows that it can very likely be explained without the need to invoke far-fetched mechanisms. As usual, Darwinian evolution is the key ingredient that ultimately makes sense of the biology.

References

1. Riggs, A.D. (1975). X-inactivation, differentiation and DNA methylation. Cytogenet. Cell Genet. *14*, 9 - 25.
2. Holliday, R., and Pugh, J.E. (1975). DNA modification mechanisms and gene activity during development. Science *186*, 226 - 232.
3. Edwards, J.R., Yarychkivska, O., Boulard, M., and Bestor, T.H. (2017). DNA methylation and DNA methyltransferases. Epigenetics Chromatin *10*, 23.
4. Sharif, J., Muto, M., Takebayashi, S., Suetake, I., Iwamatsu, A., Endo, T.A., Shinga, J., Mizutani-Koseki, Y., Toyoda, T., Okamura, K., et al. (2007). The SRA protein Np95 mediates epigenetic inheritance by recruiting Dnmt1 to methylated DNA. Nature *450*, 908-912.
5. Bostick, M., Kim, J.K., Esteve, P.O., Clark, A., Pradhan, S., and Jacobsen, S.E. (2007). UHRF1 plays a role in maintaining DNA methylation in mammalian cells. Science *317*, 1760-1764.
6. Lister, R., Pelizzola, M., Dowen, R.H., Hawkins, R.D., Hon, G., Tonti-Filippini, J., Nery, J.R., Lee, L., Ye, Z., Ngo, Q.M., *et al*. (2009). Human DNA methylomes at base resolution show widespread epigenomic differences. Nature *462*, 315-322.
7. Catania, S., Dumesic, P.A., Pimentel, H., Nasif, A., Stoddard, C.I., Burke, J.E., Diedrich, J.K., Cook, S., Shea, T., Geinger, E., et al. (2020). Evolutionary persistence of DNA methylation for millions of years after ancient loss of a *de novo* methyltransferase. Cell *180*, 263-277 e220.
8. Huff, J.T., and Zilberman, D. (2014). Dnmt1-independent CG methylation contributes to nucleosome positioning in diverse eukaryotes. Cell *156*, 1286-1297.

9. Liu, O.W., Chun, C.D., Chow, E.D., Chen, C., Madhani, H.D., and Noble, S.M. (2008). Systematic genetic analysis of virulence in the human fungal pathogen Cryptococcus neoformans. Cell *135*, 174-188.

10. Law, J.A., and Jacobsen, S.E. (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nat. Rev. Genet. *11*, 204-220.

11. Yadav, V., Sun, S., Billmyre, R.B., Thimmappa, B.C., Shea, T., Lintner, R., Bakkeren, G., Cuomo, C.A., Heitman, J., and Sanyal, K. (2018). RNAi is a critical determinant of centromere evolution in closely related fungi. Proc. Natl. Acad. Sci. USA *115*, 3108-3113.

12. Selker, E.U. (1999). Gene silencing: repeats that count. Cell *97*, 157-160.

13. Colot, V., Maloisel, l.L., and Rossignol, J.-L. (1996). Interchromosomal transfer of epigenetic states in Ascobolus: transfer of DNA methylation is mechanistically related to homologous recombination. Cell *86*, 855-864.

Wellcome Centre for Cell Biology, University of Edinburgh, Edinburgh, EH9 3BF, UK.

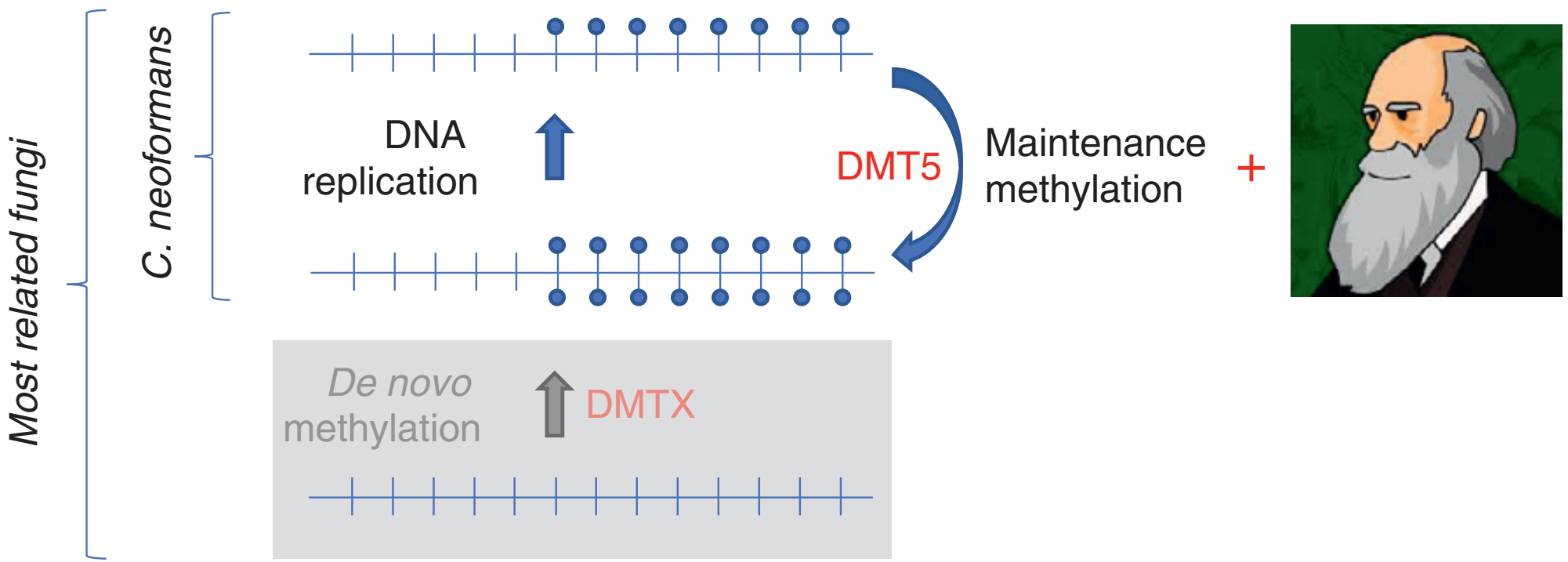
Correspondence: a.bird@ed.ac.uk

Figure 1. **(Au: Please add figure title)**

In many fungi, the *de novo* DNA methyltransferase DMTX is able to add methyl groups (blue circles) to cytosines in the self-complementary dinucleotide CG (vertical strokes). Following DNA replication, symmetrically methylated CGs become hemi-methylated. By specifically completing hemi-methylated CGs, while ignoring non-methylated CGs, the maintenance methyltransferase DMT5 restores the original DNA methylation pattern. *C. neoformans* is unusual in possessing DMT5 without any recognisable DMTX. The extraordinary constancy of its methylation pattern over millions of years is partly explained by the unprecedented efficiency of DMT5, but also requires Darwinian selection against individuals in which DNA methylation is excessively depleted.

In Brief

DNA methylation in a fungal pathogen has persisted for millions of years without the enzyme that can efficiently add methyl groups *de novo*. This spectacular example of 'epigenetic' inheritance is explained by a super-efficient maintenance enzyme plus natural selection.



Current Biology